REMARKS

The Examiner has twice rejected claims 1-7 as obvious over Watson (Biotechniques, 1997) and Ellidge (US5851808), in view of Stahl (Biotechniques, 1993). A notice of appeal, pre-appeal brief, and request for pre-appeal review are filed herewith.

Missing element not found in the cited art

None of the cited art shows "simultaneously removing and circularizing" assembled PCR fragments from a solid support with a recombinase. In the absence of these missing elements, the obviousness rejection cannot be maintained.

Competent declaratory evidence establishes no reasonable expectation of success

The Declaration of Dr. George N. Bennett, previously submitted, identifies several reasons why one of ordinary skill in the art would not have thought recombination on a solid support predictable or obvious. One important reason relates to the topology of the DNA attached to the solid support:

> Prior to the present invention, the ability of the CRE protein to function on immobilized DNA was unknown. The use of immobilized DNA for the *Cre/lox* recombination was not thought possible because immobilized DNA has a different topological structure than either native DNA *in vivo* or purified DNA *in vitro*. The *Cre/lox* reaction changes the topological structure of the DNA substrate. A DNA structure tethered to a solid support might not undergo the conformational changes required for recombination. One of ordinary skill in the art would have thought *Cre/lox* recombination was inhibited or impossible on a solid support.

DNA topology is clearly affected by binding to a solid support (as well as by its linear versus various circular forms), which in turn affects recombinase activity. This is well known in the art.[i] Thus, even if there was a suggestion in the art to modify the prior art by conducting the reactions on a solid support (and there is none because Stahl only performs ligase reactions on a solid support), there is no reasonable expectation of success where **topology is known to be critical to recombinase function**. A recombinase is **not** a ligase—the assembly of DNA on

solid support using ligase per Stahl is **not** analogous and cannot be used to extrapolate success for this unique application of recombinase.

The Examiner has provided no competent evidence

Applicants have requested the Examiner substantiate any assertions that a recombinase can reasonably be expected to function on a solid support. However, the Examiner has failed to provide any declaratory evidence, instead, making only conclusory statements that Applicants declaratory evidence is "unpersuasive," and making unsubstantiated claims about what might be reasonably expected by a recombinase.

Examiner's personal knowledge or mere argument is **not** competent evidence and is not sufficient to rebut Applicants evidence. *Fiers v. Revel*, 984 F.2d 1164 (Fed. Cir. 1993) (holding that "the Board did not err in determining that Fiers presented no convincing evidence" where applicant only showed "argument ... 'unsupported by competent evidence, entitled to little or no weight and ... unpersuasive in any event.'"); *In re Juillard*, 476 F.2d 1380 (C.C.P.A.) ("arguments cannot take the place of evidence").

Examiner is again respectfully requested to fully articulate the rationale in **proper evidentiary form** (e.g., a declaration) according to MPEP 2144.03,[1] so that Applicants may properly rebut same.

Examiner must consider competent declaratory evidence

The Examiner must consider competent evidence if provided. Applicants have provided competent evidence showing non-obviousness and Examiner has not countered with any competent evidence, therefore the obviousness rejection cannot be maintained. *In Re John B. Sullivan, et al.* (Fed. Cir. 2007) ("The claimed composition cannot be held to have been obvious if **competent evidence** rebuts the prima facie case of obviousness.").

[1] MPEP 2144.03 ("If the examiner is relying on personal knowledge to support the finding of what is known in the art, the **examiner must provide an affidavit or declaration setting forth specific factual statements and explanation to support the finding**. See 37 CFR 1.104(d)(2).") (emphasis added).

CONCLUSION

None of the cited art shows "simultaneously removing and circularizing" assembled PCR fragments from a solid support with a recombinase. The missing elements cannot be pulled from thin air, but must be properly substantiated. In the absence of competent evidence supplying the missing elements (and with competent evidence showing non-obviousness because there is no reasonable expectation of success), the claims have not been shown to be obvious and are in condition for allowance.

The Applicants respectfully request the Examiner contact them if there are any questions or procedures that need to be addressed. Appeal fees are provided and no additional fees are believed to be due for this response. However, should there be any additional fees required, please charge such additional fees to Deposit Account No. 50-3420 (reference 31175413-002002 MDB).

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Respectfully submitted,

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[i] There are many papers indicating that DNA topology is critical in the function and outcome of recombinase reactions. A few papers are highlighted herein for convenience, but this feature of recombinase activity is well known in the art:

Kilbride EA, et al., Determinants of product topology in a hybrid Cre-Tn3 resolvase site-specific recombination system, J Mol Biol. 355(2):185-95 (2006) (**Many natural DNA site-specific recombination systems achieve directionality and/or selectivity by making recombinants with a specific DNA topology. This property requires that the DNA architecture of the synapse and the mechanism of strand exchange are both under strict control.** Previously we reported that Tn3 resolvase-mediated synapsis of the accessory binding sites from the Tn3 recombination site res can

impose topological selectivity on Cre/loxP recombination. Here, we show that the topology of these reactions is profoundly affected by subtle changes in the hybrid recombination site les. Reversing the orientation of loxP relative to the res accessory sequence, or adding 4 bp to the DNA between loxP and the accessory sequence, can switch between two-noded and four-noded catenane products. By analysing Holliday junction intermediates, we show that the innate bias in the order of strand exchanges at loxP is maintained despite the changes in topology. We conclude that a specific synaptic structure formed by resolvase and the res accessory sequences permits Cre to align the adjoining loxP sites in several distinct ways, and that resolvase-mediated intertwining of the accessory sequences may be less than has been assumed previously.").

Vetcher AA, et al., DNA topology and geometry in Flp and Cre recombination, J Mol Biol. 2006 Apr 7;357(4):1089-104 (2006) ("The Flp recombinase of yeast and the Cre recombinase of bacteriophage P1 both belong to the lambda-integrase (Int) family of site-specific recombinases. These recombination systems recognize recombination-target sequences that consist of two 13bp inverted repeats flanking a 6 or 8bp spacer sequence. **Recombination reactions involve particular geometric and topological relationships between DNA target sites at synapsis**, which we investigate using nicked-circular DNA molecules. Examination of the tertiary structure of synaptic complexes formed on nicked plasmid DNAs by atomic-force microscopy, in conjunction with detailed topological analysis using the mathematics of tangles, shows that only a limited number of recombination-site topologies are consistent with the global structures of plasmids bearing directly and inversely repeated sites. The tangle solutions imply that there is significant distortion of the Holliday-junction intermediate relative to the planar structure of the four-way DNA junction present in the Flp and Cre co-crystal structures. Based on simulations of nucleoprotein structures that connect the two-dimensional tangle solutions with three-dimensional models of the complexes, we propose a recombination mechanism in which the synaptic intermediate is characterized by a non-planar, possibly near-tetrahedral, Holliday-junction intermediate. Only modest conformational changes within this structure are needed to form the symmetric, planar DNA junction, which may be characteristic of shorter-lived intermediates along the recombination pathway.").

Grainge I, et al., Symmetric DNA sites are functionally asymmetric within Flp and Cre site-specific DNA recombination synapses, J Mol Biol. 320(3):515-27 (2002) ("**Flp and Cre-mediated recombination on symmetrized FRT and loxP sites, respectively, in circular plasmid substrates yield both DNA inversion and deletion.** However, upon sequestering three negative supercoils outside the recombination complex using the resII-resIII synapse formed by Tn3 resolvase and the LER synapse formed by phage Mu transposase in the case of Flp and Cre, respectively, the reactions are channeled towards inversion at the expense of deletion. The inversion product is a trefoil, its unique topology being conferred by the external resolvase or LER synapse. Thus, Flp and Cre assign their symmetrized substrates a strictly antiparallel orientation with respect to strand cleavage and exchange. These conclusions are supported by the product profiles from tethered parallel and antiparallel native FRT sites in dilution and competition assays. Furthermore, **the observed recombination bias favoring deletion over inversion in a nicked circular substrate** containing two symmetrized FRT sites is consistent with the predictions from Monte Carlo simulations based on antiparallel synapsis of the DNA")

Crisona NJ, et al., The topological mechanism of phage lambda integrase, J Mol Biol. 18;289(4):747- 75 (1999) ("**Bacteriophage lambda integrase (Int) is a versatile site-specific recombinase. In concert with other proteins, it mediates phage integration into and excision out of the bacterial chromosome. Int recombines intramolecular sites in inverse or direct orientation or sites on separate DNA molecules. This wide spectrum of Int-mediated reactions has, however, hindered our understanding of the topology of Int recombination.** By systematically analyzing the topology of Int reaction products and using a mathematical method called tangles, we deduce a unified model for Int recombination. We find that, even in the absence of (-) supercoiling, all Int reactions are chiral, producing

one of two possible enantiomers of each product. We propose that this chirality reflects a right-handed DNA crossing within or between recombination sites in the synaptic complex that favors formation of right-handed Holliday junction intermediates. We demonstrate that the change in linking number associated with excisive inversion with relaxed DNA is equally +2 and -2, reflecting two different substrates with different topology but the same chirality. Additionally, we deduce that integrative Int recombination differs from excisive recombination only by additional plectonemic (-) DNA crossings in the synaptic complex: two with supercoiled substrates and one with relaxed substrates. **The generality of our results is indicated by our finding that two other members of the integrase superfamily of recombinases, Flp of yeast and Cre of phage P1, show the same intrinsic chirality as lambda Int**.").

Kilbride E, et al., Topological selectivity of a hybrid site-specific recombination system with elements from Tn3 res/resolvase and bacteriophage P1 loxP/Cre. J Mol Biol. 289(5):1219-30 (1999) ("In order to investigate the functions of the parts of the Tn 3 recombination site res, we created hybrid recombination sites by placing the loxP site for Cre recombinase adjacent to the "accessory" resolvase-binding sites II and III of res. The efficiency and product topology of in vitro recombination by Cre between two of these hybrid sites were affected by the addition of Tn 3 resolvase. The effects of resolvase addition were dependent on the relative orientation and spacing of the elements of the hybrid sites. **Substrates with sites II and III of res close to loxP gave specific catenated or knotted products (four-noded catenane, three-noded knot) when resolvase and Cre were added together. The product topological complexity increased when the length of the spacer DNA segment between loxP and res site II was increased. Similar resolvase-induced effects on Cre recombination product topology were observed in reactions of substrates with loxP sites adjacent to full res sites.** The results demonstrate that the res accessory sites are sufficient to impose topological selectivity on recombination, and imply that intertwining of two sets of accessory sites defines the simple catenane product topology in normal resolvase-mediated recombination. They are also consistent with current models for the mechanism of catalysis by Cre.").